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(54) Title: DNA ENCODING THE PROSTATE-SPECIFIC MEMBRANE ANTIGEN-LIKE GENE AND USES THEREOF

(57) Abstract

The present invention discloses a new gene, termed PSMA-like, that is very similar to the prostate-specific membrane antigen (PSMA) gene and cross-reacts with current detection methods for PSMA. The present invention also provides for a method of distinguishing the PSMA and PSMA-like mRNAs and/or proteins for diagnostic and therapeutic strategies that desire specific targeting of either the PSMA or PSMA-like gene.

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DNA ENCODING THE PROSTATE-SPECIFIC MEMBRANE ANTIGEN-LIKE GENE AND USES THEREOF

BACKGROUND OF THE INVENTION

Cross-reference to Related Application

This non-provisional patent application claims benefit of provisional patent application U.S. Serial number 60/128,839, filed April 9, 1999, now abandoned.

Federal Funding Legend

This invention was produced in part using funds obtained through grant DK/CA47650 from NIDDK/NCI. Consequently, the federal government has certain rights in this invention.

Field of the Invention

The present invention relates generally to the field of cell biology. More specifically, the present invention relates to the prostate-specific membrane antigen-like gene and uses thereof.

Description of the Related Art

Prostate cancer is the leading cause of cancer and second leading cause of cancer death among American males. Although

prostate tumors in the initial stages are slow growing and can be treated by radical prostatectomy and hormone deprivation, once the tumor is hormone refractory and/or has metastasized, there are few options for the patient. The major current biomarker for this disease is prostate specific antigen (PSA), however PSA is of limited value for assessing patients with disseminated disease as it is down-regulated under conditions of low androgens, and these patients undergo androgen-ablative therapy. More markers for prostate cancer are needed that have increased effectiveness over those currently used for clinical diagnosis and patient management, as well as for future therapeutic targets of this disease.

Prostate specific membrane antigen (PSMA) is an ideal potential target for use in determining patient management, and therapeutic strategies against prostate cancer. The prostate specific membrane antigen is highly expressed in virtually 100% of prostate cancers and, in contrast to PSA, the prostate specific membrane antigen is further upregulated under conditions of androgen deprivation. Furthermore, in the normal prostate, alternative splicing of prostate specific membrane antigen mRNA produces a truncated form of the protein (which has been designated PSM') that is missing the intracellular and transmembrane domains, and as such, this form is localized to the cytosol [5]. At some stage during tumor initiation or progression, there is a change in the mRNA splicing that leads to the majority of prostate specific membrane antigen transcripts comprising the transmembrane domain, thereby producing a 750 amino acid membrane-bound protein (unlike PSA, which is secreted into the circulatory system), the majority of which is located extracellularly and is readily available for therapeutic

targeting, clinical imaging or other diagnostic-type assays [5]. Prostate specific membrane antigen is already used clinically as the target of the imaging agent ProstaScint, and is the focus of a number of therapeutic strategies in development.

The known functions of the prostate specific membrane antigen carboxypeptidase are as an NAALadase and folate hydrolase. Expression of prostate specific membrane antigen is largely confined to the prostate gland, although expression can also be detected in the duodenum, brain, salivary gland, kidney, and colon [2,6]. In prostate cancer, enhanced expression of prostate specific membrane antigen correlates with increasing grade of tumor [7].

Additionally, it now seems that therapeutic targeting of the prostate specific membrane antigen molecule may have additional advantages, since prostate specific membrane antigen expression has been found in the endothelial cells of tumor neovasculature of almost all types of tumors examined to date, including bladder, renal, breast and lung carcinomas [1,6]. No prostate specific membrane antigen expression has been found in any kind of normal established non-neovasculature. As such, a therapeutic approach targeted at prostate specific membrane antigen could have broad implications for the treatment of many types of solid tumors, and several groups are now attempting to utilize prostate specific membrane antigen as a treatment target.

However, although prostate specific membrane antigen is very highly expressed in normal prostate (PSM'; the cytosolic form) and in cancer of the prostate (PSMA; the membrane bound form), there are other tissues in the body that express low levels of prostate specific membrane antigen or a similar mRNA, including kidney,

proximal small intestine and brain [4]. This mRNA could either be due to expression of the prostate specific membrane antigen gene, or another related gene such as the PSMA-like gene. Furthermore, one of the major enzymes involved in neurotransmission in the brain is NAALADase, which has the same enzymatic characteristics as prostate specific membrane antigen [7]. Accordingly, it is important to be able to distinguish between prostate- or cancer-derived prostate specific membrane antigen and PSMA-like mRNA from other tissues if prostate specific membrane antigen is going to be used as a clinical marker via techniques like RT-PCR or for therapeutic strategies, which, for example, may use antibodies.

Fluorescent *in situ* hybridization (FISH) mapping using prostate specific membrane antigen cDNA as a probe indicates that there may be two very similar genes both residing on chromosome 11 [8]. Both genes have been mapped against a human-hamster radiation hybrid panel and determined that one of the genes resides on chromosome 11p11.2, while the other gene resides on chromosome 11q14.3 [9]. It was recently determined that the gene on chromosome 11p11.2 is the PSMA gene originally cloned from the prostatic cancer cell line LNCaP [9], while a highly conserved duplication of the PSMA gene, including at least some intronic sequences, is located on chromosome 11q, a region which is known to have been duplicated to chromosome 11p an estimated 22 million years ago [16,17]. Therefore, the so-called non-prostatic expression of the prostate specific membrane antigen gene is due to expression of another highly similar, but distinct gene, herein designated the PSMA-like gene, arising from the aforementioned gene duplication. Tumor targeting for therapeutic approaches or clinical assays relies

on the specificity of the marker targeted. As the prostate specific membrane antigen and PSMA-like genes have a common ancestral gene and only diverged from each other 22 million years ago [9], it is likely that the two genes are extremely similar to each other both in sequence and in function.

The prior art is deficient in means of distinguishing between the prostate specific membrane antigen gene and the PSMA-like gene, and their respective protein products. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

Prostate specific membrane antigen is a 100 kD type II transmembrane protein with folate hydrolase and NAALADase activity. Prostate specific membrane antigen is highly expressed in prostate cancer and the vasculature of most solid tumors, and is currently the target of a number of diagnostic and therapeutic strategies. PSMA is also expressed in the brain, and is involved in conversion of the major neurotransmitter, NAAG (n-acetyl-aspartyl glutamate) to NAA and free glutamate, the levels of which are disrupted in several neurological disorders including multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease and schizophrenia.

The prostate specific membrane antigen gene (having the nucleotide sequence shown in SEQ ID No. 3) encoding prostate specific membrane antigen was recently mapped to 11p11.2, and a gene homologous (PSMA-like), but not identical, to prostate specific membrane antigen was mapped to chromosome 11q14.3, which was

subsequently mapped to the schizophrenia disorder type II locus. The mRNA tissue distribution pattern of the prostate specific membrane antigen gene and PSMA-like gene was examined using assays that specifically distinguish between the two genes by exploiting single base coding differences. Results indicate that the PSMA-like gene is expressed, as determined by RT-PCR, RNase protection assay, or using specific primers, and has a tissue distribution differing from that of the PSMA gene.

The present invention characterizes the differences between the prostatic and non-prostatic forms of prostate specific membrane antigen at the nucleic acid level, the protein level and functional level. The ability to distinguish between the PSMA and PSMA-like genes is essential for the utility of prostate specific membrane antigen, both as a prostate cancer marker and as a therapeutic target.

In one embodiment of the present invention, there is provided an isolated DNA fragment encoding a mammalian PSMA-like protein selected from the group consisting of (a) an isolated DNA fragment which encodes a PSMA-like protein; (b) an isolated DNA fragment which hybridizes to the isolated DNA fragment of (a) and which encodes a PSMA-like protein; and (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) in codon sequence due to the degeneracy of the genetic code, and which encodes a PSMA-like protein. Preferably, the DNA fragment has the sequence shown in SEQ ID No. 1 or fragments thereof, and the PSMA-like protein has the amino acid sequence shown in SEQ ID No. 2 or fragment thereof.

In another embodiment of the present invention, there is provided an isolated and purified PSMA-like protein coded for by DNA selected from the group consisting of (a) isolated DNA which encodes a PSMA-like protein; (b) isolated DNA which hybridizes to the isolated DNA of (a) and which encodes a PSMA-like protein; and (c) isolated DNA differing from the isolated DNAs from (a) and (b) in codon sequence due to the degeneracy of the genetic code, and which encodes a PSMA-like protein. Preferably, the PSMA-like protein has an amino acid sequence shown in SEQ ID No. 2 or fragments thereof.

In still another embodiment of the present invention, there is provided a method of distinguishing PSMA gene expression from PSMA-like gene expression, comprising the steps of: (a) contacting a sample with one or more oligonucleotide primer(s) under hybridizing conditions, wherein the sample comprises RNA; (b) performing RT-PCR on the sample, thereby producing RT-PCR products; (c) contacting the RT-PCR products with an appropriate restriction enzyme, thereby producing digested RT-PCR products; and (d) analyzing the digested RT-PCR products, wherein prostate specific membrane antigen gene expression is distinguished from PSMA-like gene expression by detection of fragment size(s) in the digested RT-PCR products, wherein digested PSMA-specific RT-PCR products comprise different predicted fragment size(s) compared with digested PSMA-like-specific RT-PCR products. Preferably, the oligonucleotide primer is selected from the group consisting of SEQ ID Nos. 5-38.

In yet another embodiment of the present invention, there is provided a method of distinguishing prostate specific

membrane antigen protein from PSMA-like protein in a sample, comprising the steps of: (a) contacting the sample with at least one antibody specific for a PSMA protein and/or at least one antibody specific for a PSMA-like protein under appropriate conditions; and (b) detecting binding of the antibody or antibodies. The specificity of binding is indicative of the presence of PSMA and/or PSMA-like proteins in the sample.

In yet another embodiment of the present invention, there is provided a vector for targeted gene therapy, comprising: a promoter/enhancer region from a PSMA gene or a PSMA-like gene; and a therapeutic gene. PSMA gene promoter/enhancer targets the therapeutic gene to prostate tissues and tumor neovasculature of solid tumors; whereas PSMA-like gene promoter/enhancer targets to non-prostate tissues.

In still yet another embodiment of the present invention, there is provided a method of screening for prostate specific membrane antigen or PSMA-like ligands, comprising the steps of contacting a prostate specific membrane antigen or PSMA-like protein, or fragment thereof, with potential ligands under conditions that permit protein-protein binding; removing non-specific protein-protein binding; and eluting protein bound to the PSMA or PSMA-like protein. Typically, the eluted protein is a ligand for the PSMA or PSMA-like protein.

Also provided in another embodiment of the present invention is a method of imaging cells expressing a prostate specific membrane antigen or PSMA-like protein, comprising the steps of: administering to the cells at least one compound, wherein the compound is specifically directed towards a prostate specific

membrane antigen or PSMA-like protein and labeled with an imaging agent; and detecting the imaging agent in the cells.

Further provided in an embodiment of the present invention is a cytotoxic composition, comprising: a compound specific for either a prostate specific membrane antigen protein or fragment thereof, or a PSMA-like protein or fragment thereof; and a cytotoxic agent.

Further provided is a pharmaceutical composition comprising an antibody directed against a prostate specific membrane antigen protein and does not recognize a PSMA-like protein. Such a composition can be used for diagnosing a cancer or a neurological disorder such as schizophrenia in an individual.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The appended drawings have been included herein so that the above-recited features, advantages and objects of the invention will become clear and can be understood in detail. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and should not be considered to limit the scope of the invention.

Figure 1 shows mapping of the prostate specific membrane antigen gene to chromosome 11p. **Figure 1A** shows PCR amplification of the PSMA promoter region reported by [9]. **Figure 1B** shows amplification using primers to exon 16 of the PSMA gene. **Figure 1C** shows amplification using primers to intron 6 of the PSMA gene. Genomic is normal human DNA, the subsequent 3 lanes used human-hamster hybrid DNA containing the indicated chromosomes. Hamster refers to the parental DNA. Panels A-C clearly show exonic and intronic duplication of the PSMA gene on 11p and 11q, but only 11p contains the prostate specific membrane antigen promoter region.

Figure 2 shows specific amplification of the 11q PSMA-like gene using primers designed by sequence analysis of the 11q gene.

Figure 3 shows amplification of the 3' end of prostate specific membrane antigen or PSMA-like mRNA using cDNA-specific primers with the cDNA derived from the indicated tissues. Note the lower band (splice variant) that is only present in LNCaP and prostate cells.

Figure 4 shows the alignment between prostate specific membrane antigen protein (SEQ ID No. 4) and PSMA-like protein (SEQ ID No. 2).

Figure 5 shows the NAALADase enzymatic activity of PSMA-like protein.

DETAILED DESCRIPTION OF THE INVENTION

Prostate specific membrane antigen is expressed on the cell surface making it a useful target for both clinical and therapeutic strategies. While prostate specific membrane antigen appears to be an ideal prostate cancer marker and potential therapeutic target, there have been reports of prostate specific membrane antigen expression in non-prostatic tissues, including brain, kidney and proximal small intestine. Such expression of prostate specific membrane antigen could weaken the potential of this gene as a prostate cancer marker, or at least, produce confusing and conflicting data. However, there is reason to believe that the so-called non-prostatic expression of the prostate specific membrane antigen gene is, in fact, due to expression of a highly similar, but distinct, gene, which is designated as "PSMA-like" gene. The prostate specific membrane antigen gene has recently been mapped to human chromosome 11p11.2, and the "PSMA-like" gene to chromosome 11q14.3. Characterization of the differences between the prostatic and non-prostatic forms of prostate specific membrane antigen at the nucleic acid level, the protein level and functional level is essential for the future utility of prostate specific membrane antigen, both as a prostate cancer marker and as a therapeutic target.

The differences unique to prostate specific membrane antigen can be used to generate specific antibodies for clinical imaging or immunotherapeutic approaches, RT-PCR analysis of bodily fluids specifically for prostate- or prostate cancer-derived cells. It is also possible that the two proteins differ in their enzymatic activity in such a way that prodrugs could specifically target PSMA-expressing tissues. The present invention also provides

for analysis of the sequences in the prostate specific membrane antigen gene responsible for expression in the prostate and in prostate cancer. Comparison of the promoter and enhancer sequences from the prostate specific membrane antigen gene with the corresponding regions in the PSMA-like gene (which is not expressed in the prostate) allows elucidation of those sequences responsible for prostate-specific expression. These sequences can be used to generate tissue-specific constructs for use in gene therapy against prostate cancer.

In one embodiment of the present invention, there is provided an isolated DNA fragment encoding a mammalian PSMA-like protein selected from the group consisting of (a) an isolated DNA fragment which encodes a PSMA-like protein; (b) an isolated DNA fragment which hybridizes to the isolated DNA fragment of (a) and which encodes a PSMA-like protein; and (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) in codon sequence due to the degeneracy of the genetic code, and which encodes a PSMA-like protein. Preferably, the DNA fragment has the sequence shown in SEQ ID No. 1 or fragments thereof, and the PSMA-like protein has the amino acid sequence shown in SEQ ID No. 2 or fragment thereof.

In a preferred embodiment, there is provided a vector and/or a host cell comprising the above-disclosed DNA fragment. Further preferably, the host cell can be a bacterial cell, a mammalian cell, a plant cell or an insect cell.

In another embodiment of the present invention, there is provided an isolated and purified PSMA-like protein coded for by DNA selected from the group consisting of (a) isolated DNA which

encodes a PSMA-like protein; (b) isolated DNA which hybridizes to the isolated DNA of (a) and which encodes a PSMA-like protein; and (c) isolated DNA differing from the isolated DNAs from (a) and (b) in codon sequence due to the degeneracy of the genetic code, and which encodes a PSMA-like protein. Preferably, the PSMA-like protein has an amino acid sequence shown in SEQ ID No. 2 or fragments thereof.

In a preferred embodiment, there is provided an antibody directed against the PSMA-like protein disclosed herein.

In still another embodiment of the present invention, there is provided a method of distinguishing prostate specific membrane antigen gene expression from prostate specific membrane antigen-like gene expression, comprising the steps of: (a) contacting a sample with one or more oligonucleotide primer(s) under hybridizing conditions, wherein the sample comprises RNA; (b) performing RT-PCR on the sample, thereby producing RT-PCR products; (c) contacting the RT-PCR products with an appropriate restriction enzyme, thereby producing digested RT-PCR products; and (d) analyzing the digested RT-PCR products, wherein PSMA gene expression is distinguished from PSMA-like gene expression by detection of fragment size(s) in the digested RT-PCR products, wherein digested PSMA-specific RT-PCR products comprise different predicted fragment size(s) compared with digested PSMA-like-specific RT-PCR products. Preferably, the oligonucleotide primer is selected from the group consisting of SEQ ID Nos. 5-38. Representative restriction enzymes are *Eco*RI and *Acc*I. Additionally, restriction enzymes such as *Bsp*1286I, *Sse*9I, *Tsp*509I, *Tsp*EI, *Tsp*RI, *Bst*1107I, *Aci*I, *Msp*AI, *Nsp*BII, *Rsa*I, *Hae*III or *Ssp*I may be utilized.

Representative samples are blood cells, cells growing in culture, biopsied cells, epithelial cells, endothelial cells, urine and seminal fluid.

For example, when the oligonucleotide primers are SEQ ID No. 37 and SEQ ID No. 38, and the restriction enzyme is *Eco*RI, presence of fragment sizes of 348 nucleotides and 207 nucleotides indicates PSMA gene expression in the sample, while presence of fragment size of 555 nucleotides indicates PSMA-like gene expression in the sample. Alternatively, when the restriction enzyme is *Acc*I, presence of fragment sizes of 506 nucleotides and 49 nucleotides indicates prostate specific membrane antigen gene expression in the sample and presence of fragment sizes of 319 nucleotides, 187 nucleotides and 49 nucleotides indicates PSMA-like gene expression in the sample.

In yet another embodiment of the present invention, there is provided a method of distinguishing prostate specific membrane antigen protein from prostate specific membrane antigen-like protein in a sample, comprising the steps of: (a) contacting the sample with at least one antibody specific for a PSMA protein and/or at least one antibody specific for a PSMA-like protein under appropriate conditions; and (b) detecting binding of the antibody or antibodies. The specificity of binding is indicative of the presence of prostate specific membrane antigen and/or PSMA-like proteins in the sample. Preferably, the antibody specific for a prostate specific membrane antigen protein is specific for a region of the PSMA protein and does not cross-react with a PSMA-like protein, or alternatively, the antibody specific for a PSMA-like protein is specific for a region of the PSMA-like protein and does not

cross-react with a prostate specific membrane antigen protein. Representative means of detection are colorimetric assay, fluorescence, radioautography, nuclear medicine detection, electron microscopy, enzymatic assays, enzyme-linked immunoassays and MRI.

In yet another embodiment of the present invention, there is provided a vector for targeted gene therapy, comprising: a promoter/enhancer region from a PSMA gene or a PSMA-like gene; and a therapeutic gene. PSMA gene promoter/enhancer targets the therapeutic gene to prostate tissues and tumor neovasculature of solid tumors, whereas PSMA-like gene promoter/enhancer targets to non-prostate tissues.

In still yet another embodiment of the present invention, there is provided a method of screening for prostate specific membrane antigen or prostate specific membrane antigen-like ligands, comprising the steps of contacting a PSMA or PSMA-like protein, or fragment thereof, with potential ligands under conditions that permit protein-protein binding; removing non-specific protein-protein binding; and eluting protein bound to the PSMA or PSMA-like protein. Typically, the eluted protein is a ligand for the PSMA or PSMA-like protein.

Also provided in another embodiment of the present invention is a method of imaging cells expressing a prostate specific membrane antigen or prostate specific membrane antigen-like protein, comprising the steps of: administering to the cells at least one compound, wherein the compound is specifically directed towards a PSMA or PSMA-like protein and labeled with an imaging agent; and detecting the imaging agent in the cells. Preferably, the

compound directed towards a PSMA or PSMA-like protein is an antibody or a ligand.

Still provided in an embodiment of the present invention is a cytotoxic composition, comprising: a compound specific for either a prostate specific membrane antigen protein or fragment thereof, or a prostate specific membrane antigen-like protein or fragment thereof; and a cytotoxic agent. Preferably, the compound directed towards a PSMA or PSMA-like protein is an antibody or a ligand. Preferably, the cytotoxic agent is a radioisotope or a toxin. The antibody may be linked to the cytotoxic agent either chemically or genetically. For example, the gene encoding the antibody may be fused to the gene encoding the cytotoxic agent.

Further provided is a pharmaceutical composition comprising an antibody directed against a PSMA protein and does not recognize a PSMA-like protein. Such composition can be used for diagnosing a cancer or a neurological disorder in an individual by detecting the localization of the antibody, wherein the detection of the antibody indicates a possibility of having a cancer or a neurological disorder. Representative examples of cancer include a prostate cancer, a bladder cancer, a pancreatic cancer, a sarcoma, a melanoma, a lung cancer and a kidney cancer. A representative example of a neurological disorder is schizophrenia.

In accordance with the present invention, there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, "Molecular Cloning: A Laboratory Manual (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover

ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription and Translation" [B.D. Hames & S.J. Higgins eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984). Therefore, if appearing herein, the following terms shall have the definitions set out below.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control. An "origin of replication" refers to those DNA sequences that participate in DNA synthesis. An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another

DNA sequence. A coding sequence is "operably linked" and "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

Expression vectors containing promoter sequences which facilitate the efficient transcription and translation of the inserted DNA fragment are used in connection with the host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes which are capable of providing phenotypic selection in transformed cells. The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence. A "cDNA" is defined as copy-DNA or complementary-DNA, and is a product of a reverse transcription reaction from an mRNA transcript. An "exon" is an expressed sequence transcribed from the gene locus, whereas an "intron" is a non-expressed sequence that is from the gene locus. As used in the present invention, "exons" of PSMA-like gene are

referred to regions of genomic DNA in the PSMA-like gene that are homologous to known exons in the PSMA gene; and "introns" of PSMA-like gene are referred to the regions of genomic DNA in the PSMA-like gene that are homologous to known introns in the PSMA gene.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell. As used herein, an "enhancer", "enhancer element" or "enhancer region" is a region separate from, or included with, a promoter element that typically enhances transcription or provides specific elements necessary from proper transcription. Enhancers typically can act at a distance, and often at either the 3' or 5' end of a gene. A "cis-element" is a nucleotide sequence, also termed a "consensus sequence" or "motif", that interacts with other proteins which can upregulate or downregulate expression of a specific gene locus. A "signal sequence" can also be included with the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell and directs the polypeptide to the appropriate cellular location. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and

extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters often contain "TATA" boxes and "CAAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences. As used herein, an enhancer element or region may be included with the minimal promoter elements required for transcription, to thereby create an expression pattern very similar to the native gene(s).

The term "oligonucleotide" is defined as a molecule comprised of two or more deoxyribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide. The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally, as in a purified restriction digest, or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature,

source of primer and the method used. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

Primers are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence or hybridize therewith and thereby form the template for the synthesis of the extension product. Additionally, a single base difference in a primer, particularly at the 3' end from which extension occurs, is sufficient to allow differential hybridization of the two primers, thereby allowing selected amplification based upon a single site difference. As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to enzymes which cut double-stranded DNA at or near a specific nucleotide sequence.

"Recombinant DNA technology" refers to techniques for uniting two heterologous DNA molecules, usually as a result of *in vitro* ligation of DNAs from different organisms. Recombinant DNA molecules are commonly produced by experiments in genetic engineering. Synonymous terms include "gene splicing", "molecular

cloning" and "genetic engineering". The product of these manipulations results in a "recombinant" or "recombinant molecule".

A cell has been "transformed" or "transfected" with exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a vector or plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

As used herein, the term "host" is meant to include not only prokaryotes but also eukaryotes such as yeast, plant and animal cells. A recombinant DNA molecule or gene can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Prokaryotic hosts may include *E. coli*, *S. tymphimurium*, *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells, and more preferentially, plant cells, such as *Arabidopsis thaliana* and *Tobaccum nicotiana*.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90% or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, the coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

As used herein, "fragment", as applied to a polypeptide, will ordinarily be at least 10 residues, more typically at least 20 residues, and preferably at least 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments can be generated by methods known to those skilled in the art, e.g., by enzymatic

digestion of naturally occurring or recombinant protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment, or by chemical synthesis. The ability of a candidate fragment to exhibit characteristics of a particular enzyme (e.g., binding to a specific antibody, or exhibiting partial enzymatic or catalytic activity) can be assessed by methods described herein. Purified fragments or antigenic fragments can be used to generate new regulatory enzymes using multiple functional fragments from different enzymes, as well as to generate antibodies, by employing standard protocols known to those skilled in the art.

A standard Northern blot assay can be used to ascertain the relative amounts of mRNA in a cell or tissue obtained from transgenic tissue, in accordance with conventional Northern hybridization techniques known to those persons of ordinary skill in the art. Similarly, a dot blot procedure or an RNAse protection assay can be used to evaluate the levels of mRNA expression. Alternatively, a standard Southern blot assay may be used to confirm the presence and the copy number of the gene in transgenic systems, in accordance with conventional Southern hybridization techniques known to those of ordinary skill in the art. The Northern blot, dot blot and Southern blot use a hybridization probe, e.g. radiolabelled cDNA, either containing the full-length, single stranded DNA or a fragment of the DNA sequence at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100 consecutive nucleotides in length). The DNA hybridization probe can be labelled by any of the many different methods known to those skilled in this art.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized immunoenzymatic, colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. Furthermore, the PSMA or PSMA-like enzymes can be labeled and the endogenous activities assayed. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β-glucuronidase, β-D-glucosidase, β-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion:

EXAMPLE 1

Differentiating the PSMA gene from the PSMA-like gene

To map the human PSMA gene and resolve the controversy regarding its true location (11p versus 11q), a number of primer pairs were designed with homology to various regions of the PSMA gene, including introns. These primers were then used to amplify DNA from the NIGMS somatic cell hybrid mapping panel which consists of a hybrid containing chromosome 11, one containing chromosome 11p, one containing 11q and a hamster parental line. While the amplified regions of exon 16, intron n-o (primers used correspond to nt 54278-54536 in the PSMA genomic sequence and encompass exon 15 of the PSMA gene) and intron 6 are found on both chromosome 11p and 11q, the promoter region of the PSMA gene is only amplified from the hybrid containing chromosome 11p (see Figure 1). The fact that intron sequences are present also confirms that the gene on chromosome 11q is not a pseudogene, but in fact, a gene duplication.

Intron-based primers were then used to amplify and subsequently clone regions from the 11p and 11q genes. The existence of sequence differences between the two genes was confirmed by analysis of the corresponding regions in four normal DNA samples. Based on the number of single base differences between non-coding regions of the two genes, it is estimated that the gene duplication occurred 22 million years ago, after the divergence

of man and mouse. Taken together with data from the mouse model (i.e. only one gene corresponding to the PSMA gene family is present and maps to a region that corresponds to human chromosome 11q), it is expected that the 11q gene (the PSMA-like gene) is the ancestral gene, and therefore, is likely to be functional. The fact that the promoter region of the PSMA-like gene was not subject to duplication implies at least a differential expression pattern for the two genes, which is also supported by the fact that the mouse homologue of PSMA-like gene is not expressed in the prostate.

Based on sequence differences between the human PSMA and PSMA-like genes, primer sequences (sense, 5'-GCCTTCATTTTCAGAACATCTCATGCAT-3', SEQ ID No. 5; antisense, 5'-GTCCATATAAACTTTCAAGAATGTG-3', SEQ ID No. 6) were designed that only amplify the first intron of the PSMA-like gene on chromosome 11q (see Figure 2). These primers are used to screen a human PAC library for the PSMA-like genomic clone.

EXAMPLE 2

Evidence for a novel PSMA splice variant

As analysis of the brain and prostate PSMA and PSMA-like genes was being carried out, RT-PCR of the terminal region of the PSMA gene detected an alternate splice form of PSMA present in LNCaP cells and normal prostate that is not present in normal brain (see Figure 3). This appears to be a novel splice variant of PSMA and the expression pattern of this variant is evaluated in prostate and other tissues.

EXAMPLE 3

Screening for ligands of PSMA/PSM'

It has previously been reported that mitochrondrial aspartate-aminotransferase (mAAST) binds to PSMA, since it co-elutes with PSMA from affinity columns made with the 7E11C5.3 antibody [27]. Using a similar isolation method, co-elution with PSMA of a protein of the size expected for mAAST from LNCaP cells was demonstrated. However, this protein is apparently not a PSMA ligand, as it is also eluted from the same 7E11C5.3 affinity column that has been treated with protein lysate from non-PSMA expressing PC3 cells.

The yeast-two-hybrid system is also being used to screen for PSMA ligands. To date, six million clones have been screened from a prostate library and six different, consistently interacting clones have been identified. Significantly, one of the positive clones corresponds to Survivin, a recently cloned apoptosis inhibitor which is highly expressed in prostate tumors, but is not typically expressed in terminally differentiated adult tissues. A second clone identified in the screen corresponds to a gene whose sequence has been put in Genbank as part of the chromosome 22 sequencing project. All six clones will be subcloned into appropriate vectors for re-confirming the protein-protein interaction with PSMA in the mammalian-two-hybrid system.

EXAMPLE 4

Sequencing the PSMA-like gene

For prostate specific membrane antigen to be useful as a therapeutic and clinical target for prostate cancers, it is necessary to

be able to readily distinguish the various transcript and/or proteins from one another (i.e. PSM', PSMA and PSMA-like). Primers that specifically amplify the chromosome 11q PSMA-like gene are used to screen a human PAC library by PCR. The general insert size of a PAC is around 100 kb, and the PAC library is considered to have a three-fold coverage of the human genome. Sequence is obtained directly from the PAC using at least two primers to PCR amplify each of the 19 exons. The primers are designed using the PSMA cDNA exonic sequences. This approach ensures that every intron-exon boundary is examined.

EXAMPLE 5

Nucleotide differences between the PSMA and PSMA-like genes

Of the 19 exons in the PSMA-like gene, 18 have been sequenced. Oligonucleotide primers based upon intronic sequences of the PSMA genomic clone (GenBank Accession No. AF007544) (Table 1) were used to amplify the corresponding regions of the PSMA-like gene from somatic cell hybrids containing human chromosome 11q (i.e. Hybrids GM11936 and GM07298 from Coriell Cell Repositories, NJ). The amplified PCR products were then purified, sequenced and compared to the cDNA sequence of the PSMA gene (GenBank Accession No. M99487). The nucleotide and amino acid differences obtained for exons 2-19 are described in Table 2. In the case of exon 19, the sequence was confirmed by 3' RACE, which also confirmed that the mRNA transcript of the PSMA-like gene ends in the same place as that of the PSMA gene.

TABLE 1

Exon	Bases	Sense Primer	Sense Primer Sequence	Anti-sense Primer	Antisense Primer sequence	PCR product size expected
1	2488-2863					
2	4994-5099	4870	ctcacctaat gtcagaggta (SEQ ID No. 7)	5254	agtatagtcctcctcag atg (SEQ ID No. 8)	384
3	10726 - 10912	10630	caaagtactt ttgtgtaactctgc (SEQ ID No. 9)	11082	cataggaaagtagt tgacacgg (SEQ ID No. 10)	452
4	18275 - 18376	18157	cctgaaggat tcattcaccctc (SEQ ID No. 11)	18457	gacccttaa ttatcggctgaaca (SEQ ID No. 12)	300
5-6	24400 - 25500	24323	atgtccaaca gtccccatgcag (SEQ ID No. 13)	25593	gacatgctta gtccattgtacc (SEQ ID No. 14)	1270
7	27927 - 28020	27871	gaaccgtttg aatgaaactgag (SEQ ID No. 15)	28058	ttacccaaat agccatccatgg (SEQ ID No. 16)	187
8-9	35216 - 36281	35127	gcagatgctc aataagtgaatcc (SEQ ID No. 17)	36334	ccagcacata acagttacttgatc (SEQ ID No. 18)	1207
10	37697 - 37816	37619	tagatgctat tgagtcgtttgc (SEQ ID No. 19)	37867	aaactgagac tcagataggctg (SEQ ID No. 20)	248
11	39896 - 39978	39825	ctgggcttgg tagtgtcctggg (SEQ ID No. 21)	40045	gcttggcaaa caagtcctggctac (SEQ ID No. 22)	220

12	41911 - 41974	41792	tgtcgttaat atgggtcagctc (SEQ ID No. 23)	42035	ttaactagac tgctgctcctag (SEQ ID No. 24)	243
13	46402 - 46469	46317	tggtaggaat ttagcagtggtc (SEQ ID No. 25)	46687	gatgctacta atgggctacctc (SEQ ID No. 26)	370
14	53129 - 53220	53053	cttctggtta atggacatctag (SEQ ID No. 27)	53264	caatcccaca ctgaattcagtg (SEQ ID No. 28)	211
15			agaatggggt ttagtttaatgg (SEQ ID No. 29)		tgagtcactttt tggagtcag (SEQ ID No. 30)	
16-17	56661 - 57307	56614	ttgtaagcta tccctataagag (SEQ ID No. 31)	57393	agttcagcaa cagtcatgttag (SEQ ID No. 32)	779
18	62423 - 62515	62305	gggtggtcct gaaaccaatccc (SEQ ID No. 33)	62553	gtgatattac agaaaggagtc (SEQ ID No. 34)	248
19	64209 - 64518	64127	atccaggaat tgcagagtgctc (SEQ ID No. 35)	64586	ttcagtttta atccatagggag (SEQ ID No. 36)	459

TABLE 2

Exon # in PSMA gene	Nucleotide changes PSMA→PSMA-like	Amino acid changes PSMA→PSMA-like
2	No change	No change
3	nt 630 t→a	Thr→Thr
	nt 584 t→c	Val→Ala
	nt 594 a→t	Ala→Ala
4	nt 739 c→t	Pro→Ser
5	nt 777 c→t	Gly→Gly
	nt 787 t→c	Tyr→His
	nt 877 g→a	Gly→Arg
6	nt 948 c→t	Ser→Ser
	nt 993 t→c	Asp→Asp
	nt 1023 g→t	Gln→His
7	nt 1092 t→c	Tyr→Tyr
	nt 1103 g→a	Arg→Gln
	nt 1150 a→g	Ile→Val
8	nt 1237 c→t	Pro→Ser
9	nt 1320 a→g	Thr→Thr
10	nt 1454 t→c	Ile→Thr
11	No change	No change
12	nt 1572 g→t	Glu→Asp
13	nt 1665 g→a	Pro→Pro
	nt 1684 c→t	His→Tyr
14	No change	No change

TABLE 2 (cont.)

Exon # in PSMA gene	Nucleotide changes PSMA→PSMA-like	Amino acid changes PSMA→PSMA-like
15	No change	No change
16	nt 2099 g→a	Ser→Asn
	nt 2140 g→t	Val→Leu
17	nt 2172 g→a	Lys→Lys
	nt 2202 t→c	Ser→Ser
18	nt 2239 g→t	
	nt 2241 a→g	Val→Leu
	nt 2314 g→a	Arg→Arg
19	nt 2442 a→t	Glu→Asp
	nt 2459 a→c	Tyr→Ser
	nt 2531 a→c	No change (3' UTR)
	nt 2534 c→t	No change (3' UTR)
	nt 2562 AG is deleted in PSMA-like gene	No change (3' UTR)
	nt 2571 c→a	No change (3' UTR)
	nt 2572 g→a	No change (3' UTR)

EXAMPLE 6

Sequences of PSMA-like gene and protein

PSMA-like gene was isolated from a liver library and sequenced. The complete sequence is shown in SEQ ID No. 1, whereas the predicted amino acid sequence of PSMA-like protein is shown in SQ ID No. 2. The alignment between PSMA and PSMA-like proteins are shown in Figure 4. It seems that the PSMA-like starts transcribing in the middle of intron 6 (compared to PSMA). It therefore results in a smaller protein, which is significantly different from PSMA. The similarity of the homologous regions of the two genes is around 98% at the amino acid level. PSMA-like protein will be tested for enzyme activity.

EXAMPLE 7

Tissue distribution of the PSMA-like gene

PCR on cDNAs from various tissues was performed using the following primer sequences:

Primer 1: 5' ACAGATATGTCATTCTGGGAGGTC 3' (SEQ ID No. 37) (sense; exon10)

Primer 2: 5' ACTGTGATACAGTGGATAGCCGCT 3' (SEQ ID No. 38) (anti-sense; exon 16)

PCR was run at 94°C for 3.5 min, 94°C for 20 sec, 61°C for 20 sec, and 72°C for 50 sec for 35 cycles. The expected size after PCR amplification from both PSMA and PSMA-like RNA is 555 base pairs.

One fifth of the reaction was then digested with *Eco*RI or *Acc*I. After 1-3 hours of digestion, the product was electrophoresed

and photographed. If the product was digested with *Eco*RI and fragments of 348 and 207 nucleotides are produced, then PSMA mRNA was present in the original sample. If an undigested, single band of 555 nucleotides is present following *Eco*RI digestion, PSMA-like RNA was present in the sample. If the product was digested with *Acc*I, bands of 506 and 49 nucleotides are expected if the original sample expressed the PSMA gene, and 319, 187 and 49 nucleotides if the PSMA-like gene was expressed.

RT-PCR analysis has shown that the PSMA gene is expressed in the vasculature of almost all solid tumors examined so far (>10), including bladder cancer, pancreatic cancer, sarcomas, melanomas, lung cancer, kidney cancer, as well as the prostate. The PSMA-like gene is expressed in kidney and liver. Some tissues exhibit all bands expected, meaning that both the PSMA and PSMA-like genes are expressed.

This method can be used to amplify other regions of the PSMA and PMSA-like gene that differ in nucleotide sequence. Numerous combinations of primers are acceptable, providing the primers hybridize to both the PSMA and PSMA-like genes and amplify a region that differs between the two genes such that restriction analysis of the product will differentiate between the genes. For example, in exon 8, *Bsp*1286I restricts PSMA but not PSMA-like DNA; in exon 10, the PSMA gene, but not the PSMA-like gene, is digested by *Sse*9I, *Tsp*509I or *Tsp*EI; in exon 12, PSMA is digested by *Eco*RI, while PSMA-like is not; in exon 13, PSMA-like DNA, but not PSMA DNA, is digested by *Tsp*RI, *Acc*I or *Bst*1107I, and PSMA DNA, but not PSMA-like DNA is digested by *Aci*I, *Msp*AI, *Nsp*BII or *Rsa*I; in exon 18, PSMA is restricted by *Hae*III, while PSMA-like

DNA is digested by *Ssp*I. This list is not meant to be all inclusive, but provides numerous restriction sites specific to either the PSMA or PSMA-like gene for differential identification and analysis.

EXAMPLE 8

Differential genetic marker or restriction site polymorphism

To confirm that the *Eco*RI restriction enzyme site actually differed between the two genes and was not due to a polymorphism of the PSMA gene within the population, DNA obtained from more than 15 different people was amplified using PCR primers spanning this restriction site and subsequently digested with *Eco*RI. The presence of 3 bands after digestion indicated that all the people tested had both the PSMA and PSMA-like genes, and that those genes could be distinguished by the *Eco*RI site. This result is evidence that the *Eco*RI site is not a polymorphism, but is instead a genetic marker for distinguishing the PSMA gene from the PSMA-like gene.

EXAMPLE 9

NAALADase enzymatic activity of PSMA-like protein

The PSMA-like clone obtained from screening the liver cDNA library was excised and cloned into the pIRES-neo vector (Clontech). PC-3 cells, which do not express PSMA, PSMA-like or have NAALADase activity were then transfected with the PSMA-like-neo vector using Lipofectamine Plus (Gibco-BRL) [9]. Transfected cells stably expressing the PSMA-like gene were then selected for by growing them in 1000ug/ml Geneticin. Protein was isolated from the cell lines by lysing them in 50 mM Tris-HCl pH 7.4, 0.5% Triton

X-100. 2 µg of protein was incubated with 20 µM tritiated NAAG in a total volume of 100 µl of lysis buffer for one hour. The substrate and its cleaved by-products were separated via ion-exchange chromatography and tritiated glutamate was eluted from the column in 1 M formic acid and quantified by counting in a scintillation counter. Control experiments included C4-2 LNCaP cells (positive control) and PC-3 cells that had been transfected with the pIRES-neo vector alone.

The data shows that cells transfected with the PSMA-like vector have nearly 15 fold over that seen with the cells transfected with vector alone (background counts, Figure 5). Thus PSMA-like does have NAALADase activity, and should be taken into account when designing prodrug strategies targeting PSMA. NAALADase enzymatic activity suggests that PSMA-like may be able to be secreted to the serum/urine/seminal fluid, and that differentiating PSMA-like and PSMA proteins may make all the difference in diagnosis. For example, PSMA-like protein may be used for diagnosing neurological disorders such as schizophrenia.

The following references were cited herein:

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[7] R.E. Carter, et al, Proc. Natl. Acad. Sci. 93 (1996) 749-753.

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[10] Luthi-Carter, et al. J. Pharm. Exp. Ther. 286 (1998) 1020-1025.

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Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A DNA fragment encoding a mammalian prostate specific membrane antigen-like protein selected from the group consisting of:

(a) an isolated DNA fragment which encodes a prostate specific membrane antigen-like protein;

(b) an isolated DNA fragment which hybridizes to the isolated DNA fragment of (a) above and which encodes a prostate specific membrane antigen-like protein;

(c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a prostate specific membrane antigen-like protein.

2. The DNA fragment of claim 1, wherein said DNA fragment has the sequence shown in SEQ ID No. 1 or fragments thereof.

3. The DNA fragment of claim 1, wherein said prostate specific membrane antigen-like protein has the amino acid sequence shown in SEQ ID No. 2 or fragments thereof.

4. A vector comprising the DNA fragment of claim 1 and regulatory elements necessary for expression of the DNA in a cell.

5. The vector of claim 4, wherein said DNA fragment encodes a prostate specific membrane antigen-like protein having the amino acid sequence shown in SEQ ID No. 2 or fragments thereof.

6. A host cell transfected with the vector of claim 4, wherein said vector expresses a prostate specific membrane antigen-like protein.

7. The host cell of claim 6, wherein said cell is selected from the group consisting of a bacterial cell, a mammalian cell, a plant cell and an insect cell.

8. An isolated and purified prostate specific membrane antigen-like protein coded for by DNA selected from the group consisting of:

(a) isolated DNA which encodes a prostate specific membrane antigen-like protein;

(b) isolated DNA which hybridizes to the isolated DNA of (a) above and which encodes a prostate specific membrane antigen-like protein; and

(c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a prostate specific membrane antigen-like protein.

9. The isolated and purified prostate specific membrane antigen-like protein of claim 8, wherein said prostate

specific membrane antigen-like protein has an amino acid sequence shown in SEQ ID No. 2 or fragments thereof.

10. An antibody directed against the prostate specific membrane antigen-like protein of claim 8.

11. A method of distinguishing prostate specific membrane antigen gene expression from prostate specific membrane antigen-like gene expression in a sample, comprising the steps of:

(a) contacting the sample with one or more oligonucleotide primer(s) under hybridizing conditions, wherein said sample comprises RNA;

(b) performing RT-PCR on said sample, thereby producing RT-PCR products;

(c) contacting said RT-PCR products with an appropriate restriction enzyme, thereby producing digested RT-PCR products; and

(d) analyzing said digested RT-PCR products, wherein prostate specific membrane antigen gene expression is distinguished from prostate specific membrane antigen-like gene expression by detection of fragment size(s) in the digested RT-PCR products, wherein digested prostate specific membrane antigen-specific RT-PCR products comprise different predicted fragment size(s) compared with digested prostate specific membrane antigen-like-specific RT-PCR products.

12. The method of claim 11, wherein said oligonucleotide primer is selected from the group consisting of SEQ ID Nos. 5-38.

13. The method of claim 11, wherein said sample is selected from the group consisting of blood cells, cells growing in culture, biopsied cells, epithelial cells, endothelial cells, urine and seminal fluid.

14. The method of claim 11, wherein said restriction enzyme is selected from the group consisting of *Eco*RI, *Acc*I, *Bsp*1286I, *Sse*9I, *Tsp*509I, *Tsp*EI, *Tsp*RI, *Bst*1107I, *Aci*I, *Msp*AI, *Nsp*BII, *Rsa*I, *Hae*III and *Ssp*I.

15. The method of claim 11, wherein when said oligonucleotide primers are SEQ ID No. 37 and SEQ ID No. 38, and said restriction enzyme is *Eco*RI, presence of fragment sizes of 348 nucleotides and 207 nucleotides indicates PSMA gene expression in said sample, while presence of fragment size of 555 nucleotides indicates PSMA-like gene expression in said sample.

16. The method of claim 11, wherein when said oligonucleotide primers are SEQ ID No. 37 and SEQ ID No. 38, and said restriction enzyme is *Acc*I, presence of fragment sizes of 506 nucleotides and 49 nucleotides indicates prostate specific membrane antigen gene expression in said sample, while presence of fragment sizes of 319 nucleotides, 187 nucleotides and 49 nucleotides

indicates prostate specific membrane antigen-like gene expression in said sample.

17. A method of distinguishing prostate specific membrane antigen protein from prostate specific membrane antigen-like protein in a sample, comprising the steps of:

(a) contacting a sample with at least one antibody specific for a prostate specific membrane antigen protein and/or at least one antibody specific for a prostate specific membrane antigen-like protein under appropriate conditions; and

(b) detecting binding of said antibody or antibodies, wherein binding is indicative of the presence of prostate specific membrane antigen and/or prostate specific membrane antigen-like proteins in said sample.

18. The method of claim 17, wherein said sample is selected from the group consisting of blood cells, cells growing in culture, biopsied cells, epithelial cells, endothelial cells, urine and seminal fluid.

19. The method of claim 17, wherein said antibody specific for a prostate specific membrane antigen protein is specific for a region of said prostate specific membrane antigen protein and does not cross-react with a prostate specific membrane antigen-like protein.

20. The method of claim 17, wherein said antibody specific for a PSMA-like protein is specific for a region of said PSMA-like protein and does not cross-react with a PSMA protein.

21. The method of claim 17, wherein upon binding, said detecting is by means selected from the group consisting of a colorimetric assay, fluorescence, radioautography, nuclear medicine detection, electron microscopy, enzymatic assays, enzyme-linked immunoassays and MRI.

22. A vector for targeted gene therapy, comprising:

(a) a promoter/enhancer region from a gene selected from the group consisting of a prostate specific membrane antigen gene or a prostate specific membrane antigen-like gene; and

(b) a therapeutic gene.

23. The vector of claim 22, wherein when said promoter/enhancer region is from a prostate specific membrane antigen gene, said targeting is to regions selected from the group consisting of prostate tissues and tumor neovasculature of solid tumors, and wherein when said promoter/enhancer region is from a prostate specific membrane antigen-like gene, said targeting is to non-prostate tissues.

24. A method of screening for prostate specific membrane antigen-like ligands, comprising the steps of:

(a) contacting a prostate specific membrane antigen-like protein or fragment thereof with potential ligands under conditions that permit protein-protein binding;

(b) removing non-specific protein-protein binding; and

(c) eluting protein bound to said prostate specific membrane antigen-like protein or fragment thereof, wherein said protein bound to said prostate specific membrane antigen-like protein or fragment thereof is a ligand for said prostate specific membrane antigen-like protein.

25. A method of screening for prostate specific membrane antigen ligands, comprising the steps of:

(a) contacting a prostate specific membrane antigen protein or fragment thereof with potential ligands under conditions that permit protein-protein binding;

(b) removing non-specific protein-protein binding; and

(c) eluting protein bound to said prostate specific membrane antigen protein or fragment thereof, wherein said protein bound to said prostate specific membrane antigen protein or fragment thereof is a ligand for said prostate specific membrane antigen protein.

26. A method of imaging cells expressing a prostate specific membrane antigen-like protein, comprising the steps of:

(a) administering to the cells at least one compound directed towards a prostate specific membrane antigen-like protein, wherein said compound is labeled with an imaging agent; and

(b) detecting said imaging agent in said cells.

27. The method of claim 26, wherein said compound is selected from the group consisting of an antibody and a ligand.

28. A method of imaging cells expressing a prostate specific membrane antigen protein, comprising the steps of:

(a) administering to the cells at least one compound directed towards a prostate specific membrane antigen protein, wherein said compound is labeled with an imaging agent; and

(b) detecting said imaging agent in said cells.

29. The method of claim 28, wherein said compound is selected from the group consisting of an antibody and a ligand.

30. A cytotoxic composition, comprising:

(a) a compound specific for a prostate specific membrane antigen protein or fragment thereof, or a prostate specific membrane antigen-like protein or fragment thereof; and

(b) a cytotoxic agent.

31. The cytotoxic composition of claim 30, wherein said compound is selected from the group consisting of an antibody and a ligand.

32. The cytotoxic composition of claim 30, wherein said cytotoxic agent is selected from the group consisting of a radioisotope and a toxin.

33. A pharmaceutical composition, comprising:

(a) an antibody directed against a prostate specific membrane antigen protein, wherein said antibody does not recognize a prostate specific membrane antigen-like protein; and

(b) a carrier.

---

34. A method of diagnosing a cancer in an individual, comprising the steps of:

(a) administering the composition of claim 33 to said individual; and

(b) detecting the localization of the antibody, wherein the detection of said antibody indicates a possibility of having a cancer in said individual.

35. The method of claim 34, wherein said cancer is selected from the group consisting of a prostate cancer, a bladder cancer, a pancreatic cancer, a sarcoma, a melanoma, a lung cancer and a kidney cancer.

36. A method of diagnosing a neurological disorder in an individual, comprising the steps of:

(a) administering the composition of claim 33 to said individual; and

(b) detecting the localization of the antibody, wherein the detection of said antibody indicates a possibility of having a neurological disorder in said individual.

5

37. The method of claim 36, wherein said neurological disorder is schizophrenia.

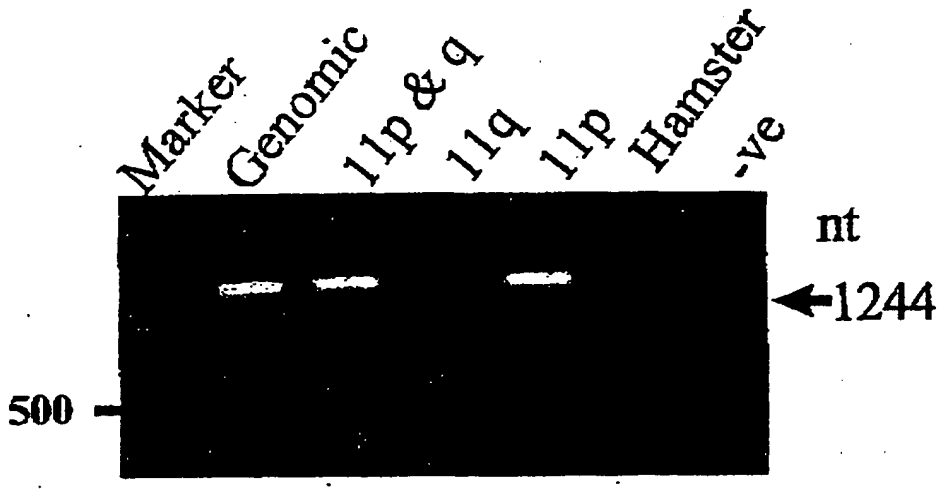
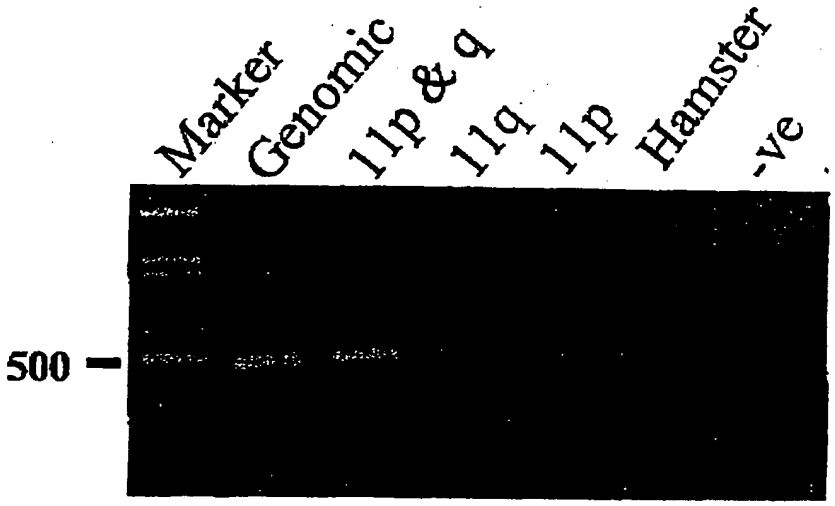


Fig. 1A

Fig. 1B

Fig. 1C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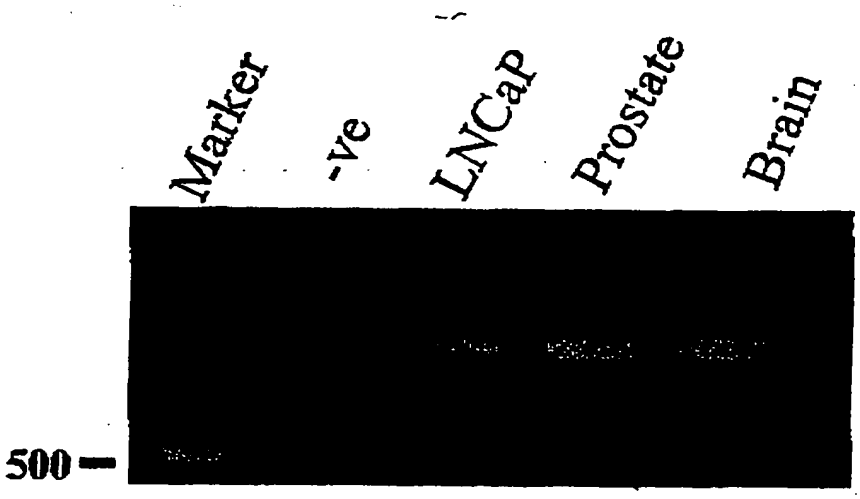
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Fig. 3

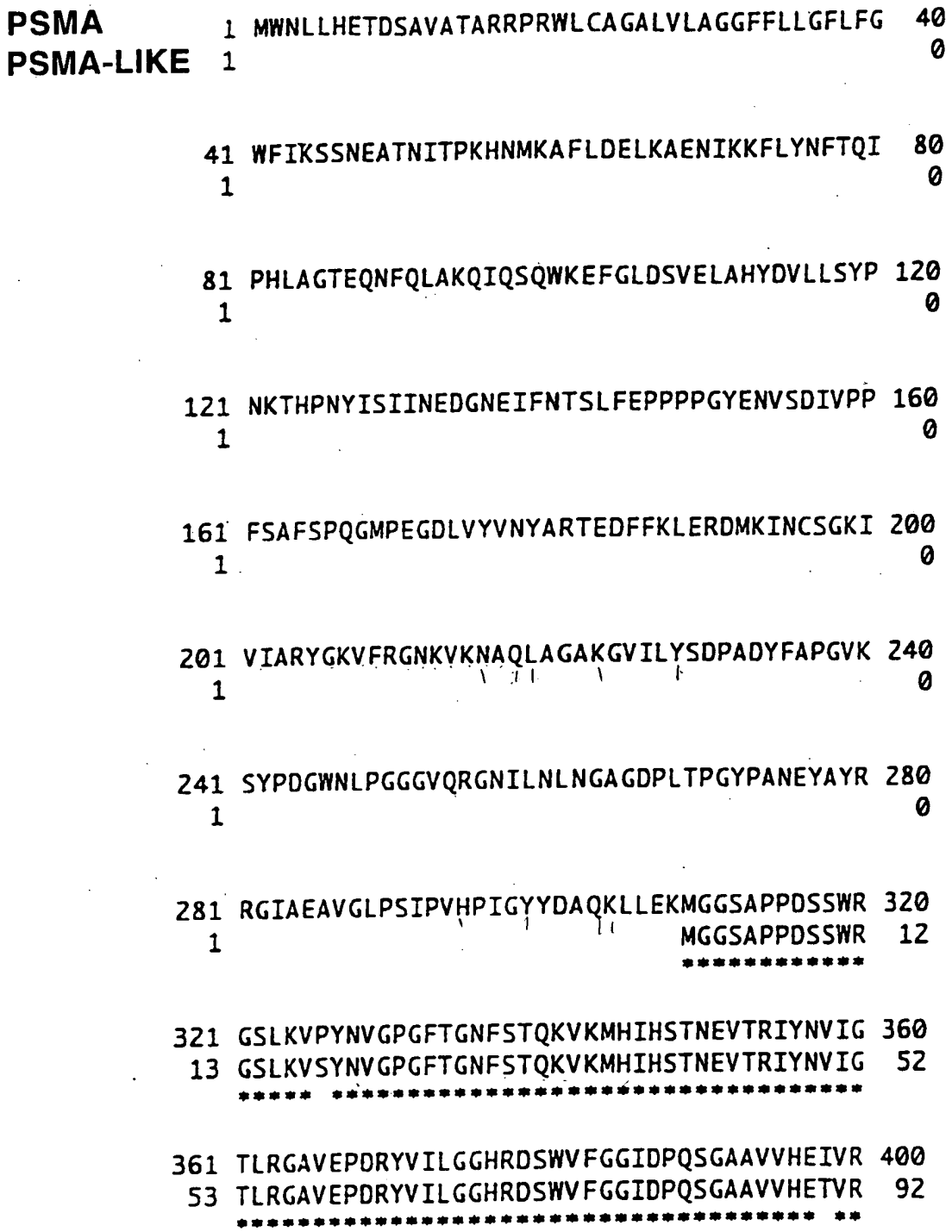
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Fig. 4-1

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Fig. 4-2

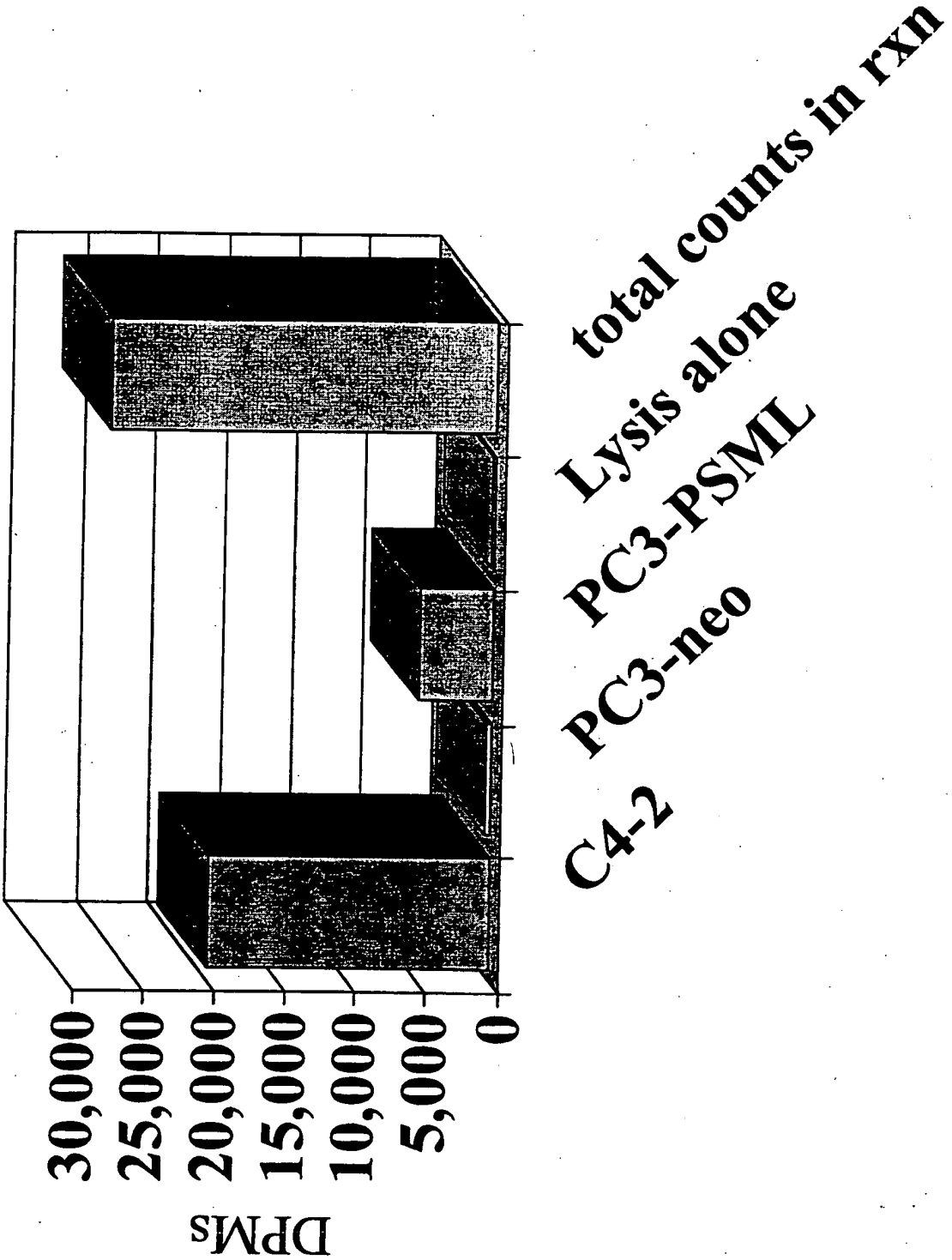
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FIG. 5

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Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu Arg Ala Phe

665 670 675

Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg His Val

680 685 690

Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser Phe

695 700 705

Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp

710 715 720

Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Tyr Val Ala

725 730 735

Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala

740 745 750

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gccttcattt tcagaacatc tcatgcat 28

SEQ 7

<210> 6
<211> 25
<212> DNA
<213> Artificial sequence
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<221> primer_bind
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<210> 7
<211> 20
<212> DNA
<213> Artificial sequence
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<221> primer_bind
<223> sense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 2)
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<211> 20
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<211> 24
<212> DNA
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<210> 10
<211> 22
<212> DNA
<213> Artificial sequence
<220>
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<223> antisense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 3)
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cataggaaag tagttgacac gg 22

<210> 11
<211> 22
<212> DNA
<213> Artificial sequence
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<223> sense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 4)
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cctgaaggat tcattcaccc tc 22

<210> 12
<211> 24
<212> DNA
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<221> primer_bind
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gacccttaa ttatcggctg aaca 24

<210> 13
<211> 22
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<212> DNA
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<210> 17
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<212> DNA
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<210> 23
<211> 22
<212> DNA
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<220>
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<210> 24
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<210> 25
<211> 22
<212> DNA
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<210> 26
<211> 22
<212> DNA
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<210> 27
<211> 22
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<223> sense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 14)
<400> 27
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<210> 28
<211> 22
<212> DNA
<213> Artificial sequence
<220>
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<223> antisense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 14)
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<210> 29
<211> 22
<212> DNA
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<223> sense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 15)
<400> 29
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<210> 30
<211> 21
<212> DNA
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<221> primer_bind
<223> antisense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exon 15)
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<210> 31
<211> 22
<212> DNA
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<221> primer_bind
<223> sense oligonucleotide primer based upon intronic sequences of the PSMA genomic clone used to amplify the corresponding regions of the PSMA-like gene (exons 16-17)
<400> 31
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<210> 32
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<212> DNA
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<400> 32
agttcagcaa cagtcatgtt ag 22

<210> 33
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<210> 35
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<213> Artificial sequence
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<220>
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<210> 37
<211> 24
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<213> Artificial sequence
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<210> 38

<211> 24
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<213> Artificial sequence
<220>
<221> primer_bind
<223> antisense primer (exon 16) used for performing PCR on cDNAs from various tissues
<400> 38
actgtgatac agtggatagc cgct 24

SEQ 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09417

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 530/350; 435/320.1, 325, 252.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/320.1, 325, 252.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,538,866 A (ISRAELI et al.) 23 July 1996 (23.07.1996), abstract.	1, 4, 6-8, 10-11, 13-14, 17-37
Y	DUMAS et al. Molecular Expression of PSMA mRNA and Protein in Primary Renal Tumors. Int. Journal of Cancer, 1999 Vol 80, pages 799-803, especially page 799.	1, 4, 6-8, 10-11, 13-14, 17-37
Y	GOOD et al. Cloning and Characterization of the Prostate-Specific Membrane Antigen Promoter, Journal of Cellular Biochemistry. September 1999, Volume 74, pages 395-405, especially page 395.	1, 4, 6-8, 10-11, 13-14, 17-37

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 JUNE 2000	05 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JENNIFER E. NICHOLS, NEE HUNT Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09417

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GRAUER et al. Identification, Purification, and Subcellular Localization of Prostate-specific Membrane Antigen PSM Protein in the LNCaP Prostatic Carcinoma Cell Line. Cancer Research. 01 November 1998, Vol. 58, No. 21, pages 4787-4789, especially page 4789.	1, 4, 6-8, 10-11, 13-14, 17-37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09417

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2-3, 5, 9, 12, and 15-16
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The claims recite SEQ ID NO's, but the sequences were not in compliance with sequence rules.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09417

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 1/00, 14/00; C12N 15/00, 15/09, 15/63, 15/70, 15/74, 5/00, 5/02, 1/12, 1/20